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# UNITED STATES ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, D.C. 20460



December 13, 1993

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

**MEMORANDUM** 

SUBJECT: Metribuzin and DADK (metribuzin metabolite) Aquatic

Invertebrate Life Cycle Tests--6(a) (2) data (D191956 and

D191547)

TO:

Walter Waldrop, PM 71

Special Review and Reregistration Division (H7508W)

FROM:

Anthony F. Maciorowski, Chief

Ecological Effects Branch

Environmental Fate and Effects Division (H7507C)

Miles, Inc. has submitted 2 aquatic invertebrate life cycle studies as 6(a)(2) data. One study (MRID #427312-01) is on the parent compound, metribuzin. This study is classified as core, with an MATC of >1.29<2.62 mg a.i./L for survival, time to first brood, length and number of offspring per adult per day. The MATC for dry weight could not be determined due to effects at all but the highest and lowest treatment levels and significant differences in the dry weights of the solvent and dilution water controls. other (MRID # 427325-01) is on DADK, a metabolite of metribuzin. This study is classified as core, with an MATC of 3.3-5.9 mg a.i./L (geometric mean MATC of 4.4 mg a.i./L). EEB has calculated preliminary, "rough-cut" estimated environmental concentrations for typical uses of metribuzin (sugarcane, potatoes, carrot, asparagus). The LOEC for survival, time to first brood, length and number of offspring for parent metribuzin was not exceeded for any of these uses. EECs for DADK could not be calculated at this time due to the lack of environmental fate data for this metabolite; therefore, EEB cannot assess the risk from this metabolite of metribuzin at the present time.

The following data requirements are **outstanding** for parent metribuzin:

71-4: Avian reproduction (TGAI)

72-4: Fish early life stage/aquatic invertebrate life cycle (TGAI)

123-1: Tier II seed germination/seedling emergence (TGAI)

123-2: Tier II aquatic plant growth (all 5 species).

The following data requirements are **reserved** for parent metribuzin: 72-5: Fish life cycle (TGAI)
72-6: Aquatic organism accumulation (TGAI)

If you have any questions on the above, please contact Kathryn Valente (308-2804).

: D191956 DP Barcode 101101 DEC | 5 |993 PC Code No

EEB Out

To:

Walter Waldrop

Product Manager 71

Special Review and Reregistration Division (H7508W)

From: Anthony F. Maciorowski, Chief

Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of ...

Reg./File # : 101101-Chemical Name : Metribuzin : <u>Herbicide</u> Type Product Product Name : Sencor Technical : Miles Inc. Company Name : Submission of Daphnia life-cycle test in Purpose support of reregistration of List A, Case No. Submitted as 6(a)(2) data. 0181. Date Due 04/01/94 Action Code 625 Reviewer K. Valente Date In 06/29/93

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)	*		72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)			122-1(B)		
71-3			72-3(C)	v <del>-</del>		122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)			123-1(B)		
71-5(A)			72-3(F)			123-2		
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)	427312-01	<b>A</b>	124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6	•		141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur

P=Partial (Study partially fulfilled Guideline but

additional information is needed

S=Supplemental (Study provided useful information but Guideline was not satisfied)

N=Unacceptable (Study was rejected)/Nonconcur

REREG CASE # 0181

DP BARCODE: D191956

CASE: 819350 SUBMISSION: S442106 DATA PACKAGE RECORD

BEAN SHEET

DATE: 06/07/93 Page 1 of 1

\* \* \* CASE/SUBMISSION INFORMATION \* \* \*

CASE TYPE: REREGISTRATION

ACTION: 625 6(A)(2) REREG. SPE. REVI

CHEMICALS: 101101 Metribuzin

ID#: 101101-

COMPANY:

PRODUCT MANAGER: 71 WALTER WALDROP 703-308-8062 ROOM: CS1 3B3
PM TEAM REVIEWER: ERIC FERIS 703-308-8048 ROOM: CS1 3G5

RECEIVED DATE: 04/09/93 DUE OUT DATE: 05/09/93

\* \* \* DATA PACKAGE INFORMATION \* \* \*

DP BARCODE: 191956 EXPEDITE: N DATE SENT: 06/07/93 DATE RET.:

CHEMICAL: 101101 Metribuzin

DP TYPE: 001 Submission Related Data Package

PATE 9/

ADMIN DUE DATE: 07/02/93 CSF: N

DATE OUT

ASSIGNED TO DIV : EFED

BRAN: EEB

SECT: IO
REVR:
CONTR:

/ / / /

PRATECTED DUE DATE

LABEL: N

04/01/94

\* \* \* DATA REVIEW INSTRUCTIONS \* \* \*

LIST A RE-REGISTRATION CHEMICAL 6(A)(2) ADVERSE EFFECTS DATA

Please review these data. Determine if the guidline requirement is satisfied and also Please make sure the DER comments on the 6(a)(2) aspects of the study.

Thanks...

\* \* \* ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION \* \* \*

DP BC BRANCH/SECTION DATE OUT DUE BACK INS CSF LABEL

DP Barcode : D191547 PC Code No **DOJ 4051** 1993

EEB Out

To:

Walter Waldrop

Product Manager 71

Special Review and Reregistration Division (H7508W)

From: Anthony F. Maciorowski, Chief

Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # :\_101101-

Chemical Name : DADK (metabolite of metribuzin)

Type Product :\_Herbicide

Product Name : None (metabolite of metribuzin) Company Name : Miles Inc.

Purpose : 6(a)(2) data review of invertebrate reproduc-

tion study. (Metribuzin: List A, Case No. 0181)

Action Code :\_625 Date Due Reviewer 11/26/93 <u>K. Valente</u> Date In 05/26/93

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation

GDLN NO	MRID NO	CAT	package contains an ever GDLN NO	MRID NO	CAT	CDINING		
71-1(A)			72-2(A)			GDLN NO	MRID NO	CAT
71-1(B)			72-2(B)		_	72-7(A)		
71-2(A)			72-3(A)			72-7(B)		
71-2(B)				<u> </u>		122-1(A)		
71-3			72-3(B)			122-1(B)	2 8	
		<del> </del>	72-3(C)			122-2		<del>                                     </del>
71-4(A)			72-3(D)			123-1(A)		+
71-4(B)	ļ		72-3(E)			123-1(B)		-
71-5(A)			72-3(F)		<del> </del>			
1-5(B)			72-4(A)		<del> </del>	123-2		
<sup>7</sup> 2-1(A)			72-4(B)			124-1		
2-1(B)		1				124-2		
2-1(C)		+	72-5			141-1		
2-1(D)			72-6			141-2		1
	atisfied Guideline)/Con-		72-4b(metabolite)	427325-01	EC	141-5		+

P=Partial (Study partially fulfilled Guideline but

additional information is needed

not satisfied)

S=Supplemental (Study provided useful information but Guideline was

N=Unacceptable (Study was rejected)/Nonconcur

DP BARCODE: D191547 REREG CASE # 0181

CASE: 819350 DATA PACKAGE RECORD DATE: 05/20/93

SUBMISSION: S441159 BEAN SHEET Page 1 of 1

\* \* \* CASE/SUBMISSION INFORMATION \* \* \*

CASE TYPE: REREGISTRATION ACTION: 625 6(A)(2) REREG. SPE. REVI

CHEMICALS: 101101 Metribuzin

ID#: 101101-COMPANY:

PRODUCT MANAGER: 71 WALTER WALDROP 703-308-8062 ROOM: CS1 3B3

PM TEAM REVIEWER: ERIC FERIS 703-308-8048 ROOM: CS1 3G5

RECEIVED DATE: 04/12/93 DUE OUT DATE: 05/12/93

\* \* \* DATA PACKAGE INFORMATION \* \* \*

DP BARCODE: 191547 EXPEDITE: N DATE SENT: 05/20/93 DATE RET.: / /

CHEMICAL: 101101 Metribuzin

DP TYPE: 001 Submission Related Data Package

ADMIN DUE DATE: 06/14/93 CSF: N LABEL: N

ASSIGNED TO DATE IN DATE OUT
DIV: EFED 5/2/93 //
BRAN: EEB 5/2/93 //
SECT: IO // //
REVR: // //

\* \* \* DATA REVIEW INSTRUCTIONS \* \* \*

DUE DATE NOV 26, 1993

LIST A RE-REGISTRATION CHEMICAL

This study is being submitted with a (180-day) review timeframe, as negotiated by the 6(a)(2) "SWAT" team. PLEASE include in the DER a discussion of 6(a)(2) reportable adverse effects. Even if you think there are no such effects, say so in the DER.

Thanks!

\* \* \* ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION \* \* \*

DP BC BRANCH/SECTION DATE OUT DUE BACK INS CSF LABEL

#### DATA EVALUATION RECORD

- DADK (Metribuzin Metabolite). CHEMICAL: 1. Shaughnessey No. 101101.
- TEST MATERIAL: DADK; Batch No. NLL 5524-5; 99.9% active 2. ingredient; white crystals.
- STUDY TYPE: 72-4. Daphnia magna Life-Cycle (21-Day 3. Renewal) Chronic Toxicity Test. Species Tested: Daphnia magna.
- Influence of DADK CITATION: Heimbach, F. 1993. 4. [Metabolite of Metribuzin] on the Reproduction Rate of Water Fleas. Report No. HBF/rDm 45. Performed by Bayer AG, Leverkusen, Bayerwerk, Germany. Submitted by Miles Inc. EPA MRID No. 427325-01.
- REVIEWED BY:
  Kathryn Valente Montague, M.S. Signature: Jakopa Valento Malague
  Biologist 5. Date: 11/24k3 Biologist EFED/EEB (7507C) U.S. EPA
- numer J. Cul 6. APPROVED BY: Signature: Norm Cook Section Head Date: EFED/EEB (7507C) U.S. EPA

Henry T. Craven, M.S. Section Head EFED/EEB-U.S. EPA

Signature:

Date:

**CONCLUSIONS:** This study is scientifically sound and meets 7. the guideline requirements for a life-cycle toxicity test using the freshwater invertebrate, Daphnia magna. Daphnid weights were not measured in this test; EPA recommends that weight measurements be used instead of or in addition to length measurements. Weight should be measured in future invertebrate life-cycle tests. Based on mean measured concentrations, the MATC for Daphnia magna exposed to DADK was >3.3 and <5.9 mg ai/l (geometric mean MATC = 4.4 mg ai/l).

- RECOMMENDATIONS: 8.
- BACKGROUND: 9.

### 10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

#### 11. MATERIALS AND METHODS:

- A. <u>Test Animals</u>: Daphnia magna were obtained from the Institute for Environmental Biology in Germany. Neonates were collected from 21-day old cultures which were maintained in test dilution water under test conditions. The daphnids were fed green algae (Scenedesmus subspicatus) and an aqueous suspension of a commercial fish food.
- B. <u>Test System</u>: The test vessels were 250-ml glass beakers which were filled with 200 ml of test solution and covered with plexiglass plates. The test solutions were renewed three times weekly.

The test solutions were not aerated and maintained at 20 ±1°C in a climatic chamber. The photoperiod was 16-hour light/8-hour dark with a light intensity of approximately 1000 lux.

The dilution water was aerated well water with a conductivity of 780-830  $\mu \rm{mhos/cm}$  and a hardness of 214 mg/l as CaCO3.

At each renewal, a stock solution (10 mg ai/l) was prepared by combining 20.1 mg in 2000 ml of test water. This stock was equivalent to the highest exposure solution. Appropriate dilutions of the stock solution were made to prepare the remaining exposure solutions.

- C. <u>Dosage</u>: Twenty-one-day, static-renewal toxicity test. Based on the results of preliminary testing, six nominal concentrations (0.32, 1.0, 1.8, 3.2, 5.6, and 10 mg ai/l) were selected for the test. A dilution water control was also included.
- D. <u>Design</u>: Five daphnids (6-24 hours old) were placed into each of four replicate vessels per treatment. At each renewal, the daphnids were fed an algal suspension and an aqueous suspension of a commercial fish food.

Mortality of the  $F_0$  daphnids was determined at each renewal. The number of young produced was determined from day 7 and at each renewal thereafter. The length of all  $F_0$  daphnids was determined at test termination.

The pH and dissolved oxygen concentration (DO) of new and old solutions were monitored in the control, lowest and highest test concentrations. The temperature was measured in one control replicate.

Samples of fresh test solutions (before the addition of food and daphnids) were collected on days 0, 9, and 16 for quantitative analysis of DADK using high pressure liquid chromatography. In addition, a test for stability of the test substance was conducted with three test concentrations. For each concentration, two containers with food and two containers without food were exposed to the test conditions. After 48 and 96 hours of exposure, the concentrations of DADK were determined.

- E. Statistics: Reproduction and growth were statistically analyzed at the  $\alpha = 0.05$  level of probability. Normality and homogeneity of variance were analyzed using Kolmogorov-Smirnov test and Bartlett's test, respectively. If the data met the assumptions of normality and homogeneity of variance, a one-way analysis of variance coupled with Dunnett's test, as necessary, was conducted. If the data did not meet the assumptions, then a nonparametric U-test (Mann-Whitney) was conducted.
- 12. REPORTED RESULTS: Mean measured concentrations of the new test solution were 0.33, 1.0, 1.9, 3.3, 5.9, and 10.0 mg ai/l which ranged from 100 to 106% of nominal concentrations (Table 17, attached). Results of the stability tests demonstrated that measured concentrations averaged 98-106% of nominal concentrations (Table 18, attached). "One can therefore work on the principle that the test animals in this study were exposed precisely to the nominal concentrations during the whole study period."

At test termination, survival at 10 mg ai/l exceeded "a mortality rate of 20%, which is supposed to be natural" (Table 3, attached).

Length and the number of offspring at 5.6 mg ai/l were statistically reduced when compared to the control (Tables 4-11, attached). The highest concentration was not included in these analyses since only 2 daphnids survived to test termination. A delay in time to first brood was observed at only the highest test concentration.

During the study, the new test solutions had a pH of 7.98-8.29 and a DO of 8.1-10.3 mg/l; the old test solutions had a

pH of 8.01-8.68 and a DO of 6.7-14.1 mg/l. The temperature in the control ranged from 19.5-20.1°C.

13. <u>STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:</u>
Based on nominal concentrations, the no-observed-effect concentration (NOEC) was 3.2 mg ai/l and the lowest-observed-effect concentration (LOEC) was 5.6 mg ai/l.

A Statement of Compliance and a Quality Assurance Statement were included in the report indicating that this study was conducted in accordance with Good Laboratory Practice Standards of OECD.

## 14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. <u>Test Procedure</u>: The test procedures were generally in accordance with the SEP, but deviated as follows:

Weight of the individual parent daphnids was not measured at test termination; EPA recommends that weight be measured at test termination.

The SEP recommends a study design which includes 7 beakers with 1 daphnid each and 3 beakers containing 5 daphnids each. The test design for this study included 4 test vessels with 5 daphnids each.

The report did not indicate whether the test daphnids were randomly assigned to the test chambers as recommended.

The DO in each concentration must be measured weekly. During this study, the DO was measured at each renewal in only three treatments.

Alkalinity, hardness, and conductivity must be measured weekly in at least one treatment and the control. During this study, hardness and conductivity were measured at each renewal only in the test dilution water. Alkalinity was not measured.

B. <u>Statistical Analysis</u>: The reviewer calculated the number of young produced per female reproductive day (using the total number of young produced and the number of female reproductive days) for analysis of effects on reproduction (Tables 4-10, attached).

The reviewer used Kruskal-Wallis test, William's test, and a one-way ANOVA coupled with Bonferroni's test to analyze the survival, reproduction (number of young

produced per female reproductive day), and length data, respectively (pages 5, 11, and 16 of printouts, attached). The survival data were analyzed as proportional survival and were arcsine squareroot transformed prior to analysis. Reproduction data were squareroot transformed prior to analysis. The results of reviewer's analyses were the same as those of the author's analyses. However, the author based the results on nominal concentrations while the reviewer used mean measured concentrations.

C. <u>Discussion/Results</u>: This study is scientifically sound and meets the guideline requirements for a static-renewal, life-cycle toxicity test using the freshwater invertebrate, *Daphnia magna*. Daphnid weights were not measured in this test. EPA prefers weight to length measurements since it is more reliable. Based on mean measured concentrations, the MATC for *Daphnia magna* exposed to DADK was >3.3 and <5.9 mg ai/l (geometric mean MATC = 4.4 mg ai/l).</p>

#### D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: N/A.
- (3) Repairability: N/A.
- 15. COMPLETION OF ONE-LINER FOR STUDY: Yes; 24 November 1993.

DADK: Survival of Exposed D.magna File: 42732501.sur Transform Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED OBSERVED	1.876	6.776 6	10.696 17	6.776 5	1.876

Calculated Chi-Square goodness of fit test statistic = Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

DADK: Survival of Exposed D.magna

File: 42732501.sur Transform: ARC SINE(SQUARE ROOT(Y))

# Shapiro Wilks test for normality

0.241

0.945

Critical W (P = 0.05) (n = 28) = 0.924Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

DADK: Survival of Exposed D.magna

File: 42732501.sur Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption. Additional transformations are useless.

TITLE:

DADK: Survival of Exposed D.magna 42732501.sur

FILE:

TRANSFORM: ARC SINE (SQUARE ROOT (Y))

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE	
1	Control	1	1.0000	1.3453	•
1	Control	2	1.0000	1.3453	W <sub>a</sub>
1	Control	3	1.0000	1.3453	* · · · · · · · · · · · · · · · · · · ·
1	Control	4	1.0000	1.3453	, <b>v.</b>
2	0.32  mg ai/l		1.0000	1.3453	
2	0.32  mg ai/l	1 2 3	1.0000	1.3453	
2	0.32  mg ai/l	3	1.0000	· 1.3453	
2	0.32  mg ai/l	4	1.0000	1.3453	
3	1.0  mg ai/l	1	1.0000	1.3453	e.
. 3	1.0  mg ai/l	2	0.8000	1.1071	
3	1.0  mg ai/l	3	0.8000	1.1071	
3	1.0  mg ai/l	4	1.0000	1.3453	
4	1.8 mg ai/l	1	1.0000	1.3453	
4	1.8  mg ai/l	1 2	0.8000	1.1071	
4	1.8  mg ai/l	3	1.0000	1.3453	
4	1.8  mg ai/l	4	1.0000	1.3453	
5	3.2  mg ai/l	1 2	0.8000	1.1071	
5	3.2 mg ai/l		1.0000	1.3453	
.5	3.2 mg ai/l	3	1.0000	1.3453	
5	3.2  mg ai/l	4	1.0000	1.3,453	
6	5.6 mg ai/l	1 '	0.8000	1.1071	
6	5.6  mg ai/l	2 3	0.8000	1.1071	
6	5.6  mg ai/l		1.0000	1.3453	
6	5.6  mg ai/l	4	0.8000	1.1071	
7	10 mg ai/l	1	0.0000	0.2255	
7	10 mg ai/l	2	0.2000	0.4636	
7	10 mg ai/l	3	0.0000	0.2255	
7	10 mg ai/l	4	0.2000	0.4636	

DADK: Survival of Exposed D.magna

File: 42732501.sur Transform: ARC SINE(SQUARE ROOT(Y))

# KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	1.345	1.000	80.000
2	0.32  mg ai/l	1.345	1.000	80.000
3	1.0  mg ai/l	1.226	0.900	56.000
4	1.8  mg ai/l	1.286	0.950	68.000
5	3.2 mg ai/l	1.286	0.950	68.000
6	5.6  mg ai/l	1.167	0.850	44.000
7	10 mg ai/l	0.345	0.100	10.000

Since Calc H > Crit H REJECT Ho: All groups are equal.

DADK: Survival of Exposed D.magna

File: 42732501.sur Transform: ARC SINE(SQUARE ROOT(Y))

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

	3 per					G]	ROT	JP						
GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	0 7 -	0 6	0 3 -	0 4 —	0 5	0,2	0			. •	
7 6 3 4 5 2	10 mg ai/l 5.6 mg ai/l 1.0 mg ai/l 1.8 mg ai/l 3.2 mg ai/l 0.32 mg ai/l Control	0.345 1.167 1.226 1.286 1.286 1.345 1.345	0.100 0.850 0.900 0.950 0.950 1.000		\ • •	\ • •	\	`	`	\				

<sup>\* =</sup> significant difference (p=0.05) . = no significant difference Table q value (0.05,7) = 3.038 SE = 5.074Table q value (0.05,7) = 3.038

File: 42732501.rep

# Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	8	12	8	0

Calculated Chi-Square goodness of fit test statistic = 4.3532 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Shapiro Wilks test for normality

0.512

0.973

Critical W (P = 0.05) (n = 28) = 0.924Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 38.92 Closest, conservative, Table H statistic = 216.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 3 Actual values ==> R (# groups) = 7, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Bartletts test for homogeneity of variance

Calculated B statistic = 7.76
Table Chi-square value = 16.81 (alpha = 0.01)

Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00 Used for Chi-square table value ==> df (#groups-1) = 6

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

DADK: No. Young/Reproductive Day of Exposed D.magna 42732501.rep TITLE:

FILE:

TRANSFORM: SQUARE ROOT(Y) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE	
1	Control	1	10.6000	3.2558	
1	Control	2	10.6000	3.2558	
1	Control	3	11.7000	3.4205	
1	Control	4	10.0000	3.1623	
2	0.32 mg ai/l	4 1 2	11.6000	3.4059	
2	0.32  mg ai/l	2	12.9000	3.5917	
2	0.32  mg ai/l	3	12.6000	3.5496	
2	0.32  mg ai/l	4	9.9000	3.1464	
<b>`</b> 3	1.0  mg ai/l	1 2	10.4000	3.2249	
3	1.0  mg ai/l	2	13.0000	3.6056	
3	1.0  mg ai/l	3	12.3000	3.5071	
3	1.0  mg ai/l	4	11.4000	3.3764	
4	1.8  mg ai/l	1	10.9900	3.3151	
4	1.8 mg ai/l	2	10.6000	3.2558	
4	1.8  mg ai/l	3	10.7000	3.2711	
4	1.8 mg ai/l	4	10.5000	3.2404	
- 5	3.2  mg ai/l	1	10.4000	3.2249	
5	3.2  mg ai/l	. 2	9.7000	3.1145	
5	3.2  mg ai/l	3	11.5000	3.3912	
5	3.2  mg ai/l	4	10.0000	3.1623	
6	5.6  mg ai/l	1 2	5.2000	2.2804	
6	5.6  mg ai/l	2	6.8000	2.6077	
6	5.6  mg ai/l	3	6.1000	2.4698	
6	5.6  mg ai/l	4	4.7000	2.1679	
7	10 mg ai/l	1	.0000	0.0000	
7	10 mg ai/l	2	0.1500	0.3873	
7	10 mg ai/l	3	0.0000	0.0000	
7	10 mg ai/l	4	0.0000	0.0000	

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1 2 3 4 5 6	Control 0.32 mg ai/l 1.0 mg ai/l 1.8 mg ai/l 3.2 mg ai/l 5.6 mg ai/l 10 mg ai/l	4 4 4 4 4 4	10.725 11.750 11.775 10.698 10.400 5.700 0.038	3.274 3.423 3.428 3.271 3.223 2.381 0.097	3.375 3.375 3.375 3.271 3.223 2.381 0.097

DADK: No. Young/Reproductive Day of Exposed D.magna File: 42732501.rep Transform: SQUARE ROOT(Y)

WILLIAMS TEST	(Isotonic	regression	model)		TABLE	2	OF	2
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IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	3.375				
0.32 mg ai/l	3.375	0.920		1.72	k = 1, v = 2
1.0  mg ai/l	3.375	0.920		1.80	k = 2, v = 2
1.8  mg ai/l	3.271	0.027		1.83	k=3, v=2
3.2 mg ai/l	3.223	0.456		1.84	k = 4, v = 2
5.6 mg ai/l	2.381	8.082	*	1.85	k=5, v=2
10 mg ai/l	0.097	28.779	*	1.85	k=6, v=2

s = 0.156

Note: df used for table values are approximate when v > 20.

DADK : Daphnia magna

TRT 1 = DILUTION WATER CONTROL

TRT 2 = 0.32 mg ai/l

TRT 3 = 1.0 mg ai/l

TRT 4 = 1.8 mg ai/l

TRT 5 = 3.2 mg ai/l

TRT 6 = 5.6 mg ai/l

		TRT	REP	LENGTH	7
CACE	4		1.0000	1.0000	4.5600
CASE CASE	1 2		1.0000	1.0000	4.5600
CASE	3		1.0000	1.0000	4.9600
CASE	4		1.0000	1.0000	5.0400
CASE	5		1.0000	1.0000	4.4800
CASE	6		1.0000	2.0000	4.5600
CASE	7		1.0000	2.0000	4.8000
CASE	. 8		1.0000	2.0000	4.7200
CASE	.9		1.0000	2.0000	5.0400
CASE	10		1.0000	2.0000	4.4800
CASE	11		1.0000	3.0000	4.6400
CASE	12		1.0000	3.0000	4.4800
CASE	13		1.0000	3.0000	4.6400
CASE	14		1.0000	3.0000	5.0400 4.6400
CASE	15		1.0000	3.0000 4.0000	4.4800
CASE	16		1.0000 1.0000	4.0000	4.2400
CASE	17		1.0000	4.0000	4.3200
CASE CASE	- 18 -19		1.0000	4.0000	4.7200
CASE	20		1.0000	4.0000	4.4800
CASE	21		2.0000	1.0000	4.4800
CASE	22		2.0000	1.0000	4.9600
CASE	23		2.0000	1.0000	4.9600
CASE	24	5 (	2.0000	1.0000	4.4800
CASE	25		2.0000	1.0000	4.9600
CASE	26		2.0000	2.0000	4.8000
CASE	27		2.0000	2.0000	4.8800
CASE	28		2.0000	2.0000	4.8800
CASE	29		2.0000	2.0000	4.8800
CASE	3.0		2.0000	2.0000	4.6400
CASE	31		2.0000	3.0000	4.9600
CASE	32		2.0000	3.0000	4.8800
CASE	33		2.0000	3.0000	4.8800
CASE	34		2.0000	3.0000	4.8000 4.9600
CASE	35		2.0000	3.0000	5.0400
CASE	36		2.0000	4.0000 4.0000	4.4000
CASE	37 38		2.0000 2.0000	4.0000	4.3200
CASE CASE	39		2.0000	4.0000	5.0400
CASE	40		2.0000	4.0000	4.4000
CASE	41		3.0000	1.0000	4.0000
CASE	42		3.0000	1.0000	4.7200
CASE	43		3.0000	1.0000	4.8800
CASE	44		3.0000	1.0000	4.8000
CASE	45		3.0000	1.0000	5.0400
CASE	46		3.0000	2.0000	4.4800
CASE	47		3.0000	2.0000	4.5600
CASE	48		3.0000	2.0000	4.8000
CASE	49		3.0000	2.0000	4.8000
CASE	50		3.0000	3.0000	4.4800
CASE	51		3.0000	3.0000	4.4800
CASE	52		3.0000	3.0000	4.8000
CASE	53		3.0000	3.0000	4.8000
CASE	54		3.0000	4.0000	4.8000
CASE	55		3.0000	4.0000	4.5600
CASE	56		3.0000	4.0000	4.6400

					• .
			3.0000	4.0000	4.8000
CASE	57				
CASE	58		3.0000	4.0000	4.7200
CASE	59		4.0000	1.0000	4.4800
			4.0000	1.0000	4.6400
CASE	60				
CASE	61		4.0000	1.0000	4.4000
CASE	62		4.0000	1.0000	4.3200
CASE	63		4.0000	1.0000	4.8800
CASE	64		4.0000	2.0000	4.2400
CASE	65		4.0000	2.0000	4.8000
			4.0000	2.0000	4.4000
CASE	66				
CASE	67		4.0000	2.0000	4.8800
CASE	68		4.0000	3.0000	5.0400
CASE	69		4.0000	3.0000	4.3200
CASE	70		4.0000	3.0000	4.7200
CASE	71		4.0000	3.0000	4.9600
CASE	72		4.0000	3.0000	4.0800
	73		4.0000	4.0000	4.1600
CASE					
CASE	74		4.0000	4.0000	4.6400
CASE	75		4.0000	4.0000	4.9600
CASE	76		4.0000	4.0000	4.8000
CASE	77	14	4.0000	4.0000	4.5600
CASE	78		5.0000	1.0000	4.6400
CASE	79		5.0000	1.0000	4.4800
	80		5.0000	1.0000	4.5600
CASE					
CASE	81		5.0000	1.0000	4.4000
CASE	82	5	5.0000	2.0000	4.8000
CASE	83		5.0000	2.0000	4.6400
CASE	84		5.0000	2.0000	4.8800
CASE	85		5.0000	2.0000	4.4800
CASE	86		5.0000	2.0000	4,4000
	87			3.0000	4.6400
CASE			5.0000	T	
CASE	88		5.0000	3.0000	4.4800
CASE	89		5.0000	3.0000	4.8800
CASE	90		5.0000	3.0000	4.6400
CASE	91		5.0000	3.0000	4.6400
CASE	92		5.0000	4.0000	4.5600
CASE	93		5.0000	4.0000	4.5600
CASE	94		5.0000	4.0000	5.0400
		*			
CASE	95		5.0000	4.0000	4.3200
CASE	96		5.0000	4.0000	4.4800
CASE	97		6.0000	1.0000	4.0800
CASE	98		6.0000	1.0000	4.1600
CASE	99		6.0000	1.0000	3.8400
CASE	100		6.0000	1.0000	3.7600
				2.0000	4.3200
CASE	101		6.0000		
CASE	102		6.0000	2.0000	4.4800
CASE	103		6.0000	2.0000	3.8400
CASE	104		6.0000	2.0000	4.0000
CASE	105		6.0000	3.0000	3.7600
CASE	106		6.0000	3.0000	4.1600
CASE	107		6.0000	3.0000	4.1600
CASE	108		6.0000	3.0000	4.3200
CASE	109		6.0000	3.0000	4.0800
CASE	110		6.0000	4.0000	4.0000
CASE	111		6.0000	4.0000	3.6000
CASE	112		6.0000	4.0000	3.7600
CASE	113		6.0000	4.0000	4.0800

DADK : Daphnia magna

THE FOLLOWING RESULTS ARE FOR:

= 1.0000 TRT

TOTAL OBSERVATIONS:

20

LENGTH

N OF CASES MINIMUM 4.2400 5.0400 MUMIXAM MEAN STANDARD DEV 0.2328

THE FOLLOWING RESULTS ARE FOR:

= 2.0000

TOTAL OBSERVATIONS:

20

LENGTH

N OF CASES 20 MINIMUM 4.3200 MAXIMUM 5.0400 4.7800 MEAN STANDARD DEV 0.2349

THE FOLLOWING RESULTS ARE FOR:

TRT

= 3.0000

TOTAL OBSERVATIONS: 18

LENGTH

N OF CASES MINIMUM 4.0000 5.0400 MAXIMUM MEAN 4.6756 STANDARD DEV 0.2283

THE FOLLOWING RESULTS ARE FOR:

TRT

= 4.0000

TOTAL OBSERVATIONS:

LENGTH

N OF CASES MINIMUM 4.0800 5.0400 MAXIMUM MEAN 4.5937 STANDARD DEV 0.2937

THE FOLLOWING RESULTS ARE FOR:

TRT

5.0000

TOTAL OBSERVATIONS:

19

LENGTH

N OF CASES MINIMUM 4.3200 5.0400 4.6063 MAXIMUM MEAN STANDARD DEV 0.1854

THE FOLLOWING RESULTS ARE FOR:

TRT

6.0000

TOTAL OBSERVATIONS:

17

LENGTH

N OF CASES 17 3.6000 MINIMUM 4.4800 MAXIMUM 4.0235 MEAN STANDARD DEV 0.2379

SUMMARY STATISTICS FOR LENGTH

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE =

3.7581 DF= 5 PROBABILITY =

0.5847

22.3225

ANALYSIS OF VARIANCE

SOURCE

SUM OF SQUARES DF MEAN SQUARE

PROBABILITY

BETWEEN GROUPS

6.3016 5

1.2603

WITHIN GROUPS

6.0412 107

0.0565

0.0000

LEVELS ENCOUNTERED DURING PROCESSING ARE:  TRT 1.0000 2.0000 3.0000 4.0000  DEP VAR: LENGTH N: 113 MULTIPLE R: 0.755 SQUARED MULTIPLE R: 0.570  AMALYSIS OF VARIANCE  SOURCE SUM-OF-SQUARES DF MEAN-SQUARE F-RATIO P  TRT 6.3178 5 1.2636 21.2050 0.0000  REP 0.2853 3 0.0951 1.5957 0.1960  TRT*REP 0.2853 3 0.0951 1.5957 0.1960  TRT*REP 0.4565 15 0.0304 0.5107 0.9287  ERROR 5.3034 89 0.0596  Post-hoc pairwise comparison of length/Bonferroni.  COL/ ROW TRT 1 1.0000 2 2.0000 3 3.0000 4 4.0000 5 5.0000 6 6.0000  USING LEAST SQUARES MEANS. POST NOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES: 1 2 3 4 5  1 0.0000 3 0.0290 -0.1070 0.0000 4 0.0510 -0.1870 -0.0800 0.0000 5 -0.0420 -0.1780 -0.0710 0.0090 0.0000 6 -0.6250 -0.7610 -0.6540 -0.5740 -0.5830  BONFERRONI ADJUSTMENT.  MATRIX OF PAIRWISE COMPARISON PROBABILITIES: 1 2 3 4 5  1 1.0000 2 1.0000 1.0000 3 1.0000 1.0000 4 1.0000 1.0000 5 1.0000 1.0000 1.0000 6 0.0000 0.2876 1.0000 1.0000 5 1.0000 0.2876 1.0000 1.0000 5 1.0000 0.3837 1.0000 1.0000 5 1.0000 0.0000 0.0000 0.0000 0.0000 6 0.0000 0.0000 0.0000 0.0000 0.0000	ANOVA	on Lengths	:		•		
1.0000 2.0000 3.0000 4.0000  DEP VAR: LENGTH N: 113 MULTIPLE R: 0.755 SQUARED MULTIPLE R: 0.570  ANALYSIS OF VARIANCE  SOURCE SUM-OF-SQUARES DF MEAN-SQUARE F-RATIO P  TRI 6.3178 5 1.2636 21.2050 0.0000  REP 0.2853 3 0.0951 1.5957 0.1960  TRI*REP 0.4565 15 0.0304 0.5107 0.9287  ERROR 5.3034 89 0.0596  Post-hoc pairwise comparison of length/Bonferroni.  COL/ ROW TRT 1 1.0000 2 2.0000 3 3.0000 4 4.0000 5 5.0000 6 6.0000  USING LEAST SQUARES MEANS. POST HOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES: 1 2 3 4 5 1 1 0.0000 6 6.0000 0.0000 6 6 0.00000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.00000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.00000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.00000 0.0000 0.0000 0.0000 0.0000 0.00000 0.0000 0.0000 0.0000 0.0000 0.00000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.00000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.00000 0.					4.0000	5.0000	6.0000
ANALYSIS OF VARIANCE  SOURCE SUM-OF-SQUARES DF MEAN-SQUARE F-RATIO P  TRI 6.3178 5 1.2636 21.2050 0.0000  REP 0.2853 3 0.0951 1.5957 0.1960  TRI*REP 0.4565 15 0.0304 0.5107 0.9287  ERROR 5.3034 89 0.0596  Post-hoc pairwise comparison of length/Bonferroni.  COL/ ROW TRI 1 1.0000 2 2.0000 3 3.0000 4 4.0000 5 5.0000 6 6.0000  USING LEAST SQUARES MEANS. POST HOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES: 1 2 3 4 5  1 0.0000 2 0.1350 0.0000 3 0.0290 -0.1070 0.0000 4 -0.0510 -0.1870 -0.0800 0.0000 5 5 -0.0420 -0.1780 -0.0710 0.0090 0.0000 6 -0.6250 -0.7610 -0.6540 -0.5740 -0.5830  6 6 0.0000  BONFERRONI ADJUSTMENT.  MATRIX OF PAIRWISE COMPARISON PROBABILITIES:  1 2 3 4 5  1 1.0000 2 3 1.0000 1.0000 3 1.0000 1.0000 3 1.0000 1.0000 3 1.0000 1.0000 3 1.0000 1.0000 3 1.0000 1.0000 3 1.0000 1.0000 3 1.0000 1.0000 3 1.0000 1.0000 5 1.0000 0.3837 1.0000 1.0000 5 1.0000 0.0000 0.0000 0.0000 0.0000	REP	1.0000	2.0000	3.0000	4.0000		
SOURCE SUM-OF-SQUARES DF MEAN-SQUARE F-RATIO P  TRT 6.3178 5 1.2636 21.2050 0.0000  REP 0.2853 3 0.0951 1.5957 0.1960  TRT*REP 0.4565 15 0.0304 0.5107 0.9287  ERROR 5.3034 89 0.0596  Post-hoc pairwise comparison of length/Bonferroni.  COL/ ROW TRT 1 1.0000 2 2.0000 3 3.0000 4 4.0000 5 5.0000 6 6.0000  USING LEAST SQUARES MEANS. POST HOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES: 1 2 3 4 5  1 0.0000 2 0.1360 0.0000 3 0.0290 0.1070 0.0000 4 0.0000 6 0.00290 0.1070 0.0000 6 0.06250 0.0710 0.0000 6 0.06250 0.07610 0.0540 0.0704 0.0090 6 0.06250 0.07610 0.0540 0.5740 0.5830  6 6 6 0.0000  BONFERRONI ADJUSTMENT. MATRIX OF PAIRWISE COMPARISON PROBABILITIES:  1 2 3 4 5  1 1.0000 2 1.0000 1.0000 3 1.0000 1.0000 3 1.0000 1.0000 3 1.0000 1.0000 3 1.0000 1.0000 1 1.0000 1.0000 3 1.0000 1.0000 1.0000 3 1.0000 1.0000 1.0000 4 1.0000 0.2876 1.0000 1.0000 5 1.0000 0.0000 0.0000 0.0000 0.0000	DEP VA	R: LENGTH	N: 11	3 MULTIPLE R:	0.755 SQUAF	RED MULTIPLE	R: 0.570
TRT 6.3178 5 1.2636 21.2050 0.0000 REP 0.2853 3 0.0951 1.5957 0.1960 TRT*REP 0.4565 15 0.0304 0.5107 0.9287 ERROR 5.3034 89 0.0596  Post-hoc pairwise comparison of length/Bonferroni. COL/ ROW TRT 1 1.0000 2 2.0000 3 3.0000 4 4.0000 5 5.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.0000 6 6.0000 6 6.0000 6 6.00000 6 6.00000 6 6.00000 6 6.00000 6 6.0000 6 6.00000 6 6.00000 6 6.00000 6 6.00000 6 6.000000			ANALYSIS	OF VARIANCE			
REP	SOURCE	SUM-O	F-SQUARES D	F MEAN-SQUARE	F-RATIO	P	
TRI*REP	TRT						
ERROR 5.3034 89 0.0596  Post-hoc pairwise comparison of length/Bonferroni. COL/ ROW TRT  1 1.0000 2 2.0000 3 3.0000 4 4.0000 5 5.0000 6 6.0000  USING LEAST SQUARES MEANS. POST HOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES: 1 2 3 4 5  1 0.0000 2 0.1360 0.0000 2 0.1360 0.0000 4 0.0510 -0.1870 -0.0800 0.0000 4 0.0510 -0.1870 -0.0800 0.0000 5 -0.0420 -0.1780 -0.0710 0.0090 0.0000 6 -0.6250 -0.7610 -0.6540 -0.5740 -0.5830  6 6 0.0000  BONFERRONI ADJUSTMENT. MATRIX OF PAIRWISE COMPARISON PROBABILITIES:  1 2 3 4 5  1 1.0000 2 1.0000 1.0000 3 1.0000 1.0000 4 1.0000 0.2876 1.0000 1.0000 5 1.0000 0.3837 1.0000 1.0000 6 0.0000 0.0000 0.0000 0.0000 0.0000 6 0.0000 0.0000 0.0000 0.0000 0.0000		D					
Post-hoc pairwise comparison of length/Bonferroni.  COL/ ROW TRT  1			7.		0.5101		
COL/ ROW TRT  1 1.0000 2 2.0000 3 3.0000 4 4.0000 5 5.0000 6 6.0000  USING LEAST SQUARES MEANS. POST HOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES: 1 2 3 4 5  1 0.0000 2 0.1360 0.0000 2 0.1360 0.0000 3 0.0290 -0.1070 0.0000 4 -0.0510 -0.1870 -0.0800 0.0000 5 -0.0420 -0.1780 -0.0710 0.0090 0.0000 6 -0.6250 -0.7610 -0.6540 -0.5740 -0.5830  6 6 6 0.0000  BONFERRONI ADJUSTMENT. MATRIX OF PAIRWISE COMPARISON PROBABILITIES:  1 2 3 4 5  1 1.0000 2 1.0000 1.0000 3 1.0000 1.0000 4 1.0000 0.2876 1.0000 1.0000 4 1.0000 0.2876 1.0000 1.0000 5 1.0000 0.0000 0.0000 0.0000 0.0000 6 0.0000 0.0000 0.0000 0.0000 0.0000	<del>, , , , , , , , , , , , , , , , , , , </del>				. •		<u> </u>
1 1.0000 2 2.0000 3 3.0000 4 4.0000 5 5.0000 6 6.0000  USING LEAST SQUARES MEANS. POST HOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES: 1 2 3 4 5  1 0.0000 2 0.1360 0.0000 3 0.0290 -0.1070 0.0000 4 -0.0510 -0.1870 -0.0800 0.0000 5 -0.0420 -0.1780 -0.0710 0.0090 0.0000 6 -0.6250 -0.7610 -0.6540 -0.5740 -0.5830  6 6 6 0.0000  BONFERRONI ADJUSTMENT. MATRIX OF PAIRWISE COMPARISON PROBABILITIES:  1 2 3 4 5  1 1.0000 2 1.0000 1.0000 3 1.0000 1.0000 4 1.0000 0.2876 1.0000 5 1.0000 0.3837 1.0000 1.0000 6 0.0000 0.0000 0.0000 0.0000 0.0000	COL/		comparison of	length/Bonter	roni.		
2 2,0000 3 3,0000 4 4,0000 5 5,0000 6 6,0000  USING LEAST SQUARES MEANS. POST HOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES: 1 2 3 4 5  1 0,0000 2 0,1360 0.0000 3 0,0290 -0.1070 0,0000 4 -0.0510 -0.1870 -0.0800 0.0000 5 -0.0420 -0.1780 -0.0710 0.0090 0.0000 6 -0.6250 -0.7610 -0.6540 -0.5740 -0.5830  6 6 0.0000  BONFERRONI ADJUSTMENT. MATRIX OF PAIRWISE COMPARISON PROBABILITIES:  1 2 3 4 5  1 1.0000 2 1.0000 1.0000 3 1.0000 1.0000 4 1.0000 0.2876 1.0000 1.0000 5 1.0000 0.0000 0.0000 0.0000 6 0.0000 0.0000 0.0000 0.0000 0.0000							
4 4.0000 5 5.0000 6 6.0000  USING LEAST SQUARES MEANS. POST HOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES: 1 2 3 4 5  1 0.0000 2 0.1360 0.0000 3 0.0290 -0.1070 0.0000 4 -0.0510 -0.1870 -0.0800 0.0000 5 -0.0420 -0.1780 -0.0710 0.0090 0.0000 6 -0.6250 -0.7610 -0.6540 -0.5740 -0.5830  6  6  6  6 0.0000  BONFERRONI ADJUSTMENT. MATRIX OF PAIRWISE COMPARISON PROBABILITIES:  1 2 3 4 5  1 1.0000 2 1.0000 1.0000 3 1.0000 1.0000 4 1.0000 0.2876 1.0000 1.0000 5 1.0000 0.3837 1.0000 1.0000 6 0.0000 0.0000 0.0000 0.0000 0.0000							
5 5.0000 6 6.0000  USING LEAST SQUARES MEANS. POST HOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES:  1 2 3 4 5  1 0.0000 2 0.1360 0.0000 3 0.0290 -0.1070 0.0000 4 -0.0510 -0.1870 -0.0800 0.0000 5 -0.0420 -0.1780 -0.0710 0.0090 0.0000 6 -0.6250 -0.7610 -0.6540 -0.5740 -0.5830  6  6  6 0.0000  BONFERRONI ADJUSTMENT. MATRIX OF PAIRWISE COMPARISON PROBABILITIES:  1 2 3 4 5  1 1.0000 2 1.0000 1.0000 3 1.0000 1.0000 4 1.0000 0.2876 1.0000 1.0000 4 1.0000 0.2876 1.0000 1.0000 5 1.0000 0.3837 1.0000 1.0000 6 0.0000 0.0000 0.0000 0.0000 0.0000	3			2.5			
## Comparison of Comparison Probabilities:    1	4						
POST HOC TEST OF LENGTH  MATRIX OF PAIRWISE MEAN DIFFERENCES:  1 2 3 4 5  1 0.0000 2 0.1360 0.0000 3 0.0290 -0.1070 0.0000 4 -0.0510 -0.1870 -0.0800 0.0000 5 -0.0420 -0.1780 -0.0710 0.0090 0.0000 6 -0.6250 -0.7610 -0.6540 -0.5740 -0.5830  6  6 0.0000  BONFERRONI ADJUSTMENT.  MATRIX OF PAIRWISE COMPARISON PROBABILITIES:  1 2 3 4 5  1 1.0000 2 1.0000 1.0000 3 1.0000 1.0000 4 1.0000 0.2876 1.0000 1.0000 5 1.0000 0.3837 1.0000 1.0000 6 0.0000 0.0000 0.0000 0.0000 0.0000	6		e y				
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	Identity of product inert ingredients.
	Identity of product impurities.
	Description of the product manufacturing process.
	Description of quality control procedures.
	Identity of the source of product ingredients.
	Sales or other commercial/financial information.
	A draft product label.
	The product confidential statement of formula.
	Information about a pending registration action.
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#### DATA EVALUATION RECORD

- Shaughnessey No. 101101. Metribuzin. CHEMICAL: 1.
- TEST MATERIAL: SENCOR Technical [4-amino-6-(1,1-2. dimethylethyl) -3-(methylthio) -1,2,4-triazin-5(4H)-one]; Batch No. 0145346/0030147; CAS No. 21087-64-9; 93% active ingredient; a white powder.
- STUDY TYPE: 72-4. Freshwater Invertebrate Life-Cycle 3. Toxicity Test. Species Tested: Daphnia magna.
- CITATION: Gagliano, G.G. and L.M. Bowers. 1993. Toxicity of SENCOR Technical to the Waterflea (Daphnia magna) Under Flow-Through Conditions. Miles Report No. 105023. Performed by Miles Incorporated, Stilwell, KS. Submitted by Miles Incorporated, Kansas City, MO. EPA MRID No. 427312-01.
- 5. REVIEWED BY: Kathryn Valente Montague, M.S. Biologist U.S. EPA EFED/EEB

Signature: 10/13/13

APPROVED BY: Norm Cook

Section Head U.S. EPA EFED/EEB

Date:

James Goodyear Contract Supervisor U.S. EPA

EFED/EEB

- CONCLUSIONS: This study is scientifically sound and meets 12.1493 7. the guideline requirements for a freshwater invertebrate life-cycle toxicity test. The MATC for survival, time to first brood, number offspring per parent per reproductive day and length is >1.29 mg/L <2.62 mg/L. The point-estimate MATC was 1.84 mg/L. Based on the reviewer's statistical analysis, there was a significant difference in dry weight at all levels compared to the solvent control; therefore, the MATC based on dry weight could not be determined.
- RECOMMENDATIONS: N/A. 8.
- 9. BACKGROUND:

# 10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

#### 11. MATERIALS AND METHODS:

- A. <u>Test Animals</u>: Daphnia magna were obtained from 19-day old adult daphnids which were cultured in-house. The cultures were maintained under test conditions (i.e., 20 ±1°C; a photoperiod of 16 hours of light per day). The daphnids were fed a minimum of three times per week a combination of green algae (Selenastrum capricornutum and/or Ankistrodesmus falcatus) and supplemented with a trout chow, yeast, and cereal leaf suspension.
- B. <u>Test System</u>: The test was conducted under flow-through conditions using a Mount-Brungs diluter. The test vessels were 1-1 borosilicate glass beakers filled with 900 ml of test solution. For test days 1-6, the flow rate to each test vessel was 250 ml every 66 minutes resulting in five volume turnovers per day. For the remainder of the test (i.e., days 7-21), the flow rate was 250 ml every 25 minutes resulting in 14 volume turnovers per day.

The test chambers were randomly positioned in a water bath. Sixteen hours of light at an intensity of 40-60 footcandles were provided each day. Thirty-minute dawn/dusk simulation periods were provided.

The dilution water was filtered spring water which was supplemented with treated (dechlorinated and filtered) city water. A representative sample of the dilution water had a pH range of 8.2-8.4, a specific conductivity of 371  $\mu$ mhos/cm, a residual chlorine of <0.003 mg/l, and a hardness and alkalinity of 177 and 135 mg/l as CaCO3, respectively.

A primary stock solution (100 g ai/l) was prepared by dissolving 54 g of test material in 500 ml of methanol.

c. <u>Dosage</u>: Twenty-one day, flow-through test. Based on results of a previous definitive study, nominal test concentrations selected were 0.32, 0.63, 1.25, 2.5, and 5.0 mg ai/l. A dilution water control and solvent control were also included. The solvent control had a methanol concentration of 50  $\mu$ l/l which was approximately equal to the amount of methanol received by the highest test concentration.

Design: Ten first-instar daphnids (<24 hours old) were randomly distributed to each of four test vessels per treatment (i.e., 40 daphnids/treatment). The loading rate was approximately 1 daphnid per 100 ml of test solution.

The daphnids were fed an algal suspension (<u>Selenastrum capricornutum</u> and/or <u>Ankistrodesmus falcatus</u>) at a minimum daily rate of 1 x 10<sup>7</sup> cells/l. A trout chow, yeast, and cereal leaf suspension was dispensed at least every third day at a rate of 0.5 ml/l.

Observations of survival, sublethal effects, and first brood of the organisms were recorded daily. After the release of first brood, observations of mortality and number of young produced were recorded every Monday, Wednesday, and Friday. At test termination, total body length and dry weight of each surviving adult were recorded.

Dissolved oxygen concentration (DO), pH, conductivity, temperature, total hardness, and alkalinity in alternating replicates of the controls, low, middle, and high concentrations were measured on days 0, 7, 14, and 21. Temperature was also monitored hourly in one centrally-located test chamber.

Samples of fresh test solutions were collected from alternating replicates (A, B, C, or D) on test days -1, 0, 7, 11, 14, and 21 for determination of SENCOR concentrations. Samples were analyzed using high pressure liquid chromatography.

E. <u>Statistics</u>: Data for the replicate vessels for each concentration were grouped together for analysis. For this study, each parameter was tested for assumptions of normality (chi-square test), for homogeneity of variance (Bartlett's test), and to determine if control and solvent control data could be pooled (t-test). If the controls could not be pooled, only solvent control data were used for further analysis.

A one-way analysis of variance (ANOVA) coupled with Dunnett's one-tailed multiple means comparison test, as necessary, was used to assess treatment effects on reproduction, growth, time to first brood, and survival. Statistical conclusions were made at 95% confidence level.

12. REPORTED RESULTS: Mean measured concentrations were 0.30, 0.65, 1.29, 2.62, and 5.74 mg ai/l (Table 2, attached). The measured concentrations ranged from 94 to 115% of nominal concentrations.

"Due to a diluter system malfunction on day 11, the daphnids in 5.74 mg/l Replicate [D] were washed into the waterbath...On day 18 all adult daphnia were washed into the waterbath when the splitter cup for the solvent control clogged and overflowed directly into Solvent Control Replicate [A]...The loss of adults from Solvent Control Replicate [A] and 5.74 mg/l Replicate [D] did not influence the ability to identify treatment related effects. In fact, the control and solvent control data was pooled for all endpoints, except for dry weight, which increased the power of the statistics."

Adult survival in the exposure levels was not significantly different from that of the control (Table 5, attached). The 21-day  $LC_{50}$  was established to be >5.74 mg ai/l.

Mean length of the control and solvent control organisms was 4.2 and 4.4 mm, respectively. Length of the surviving adults was not significantly affected at any test level when compared to the pooled control data (Table 7, attached).

Mean dry weight of the control and solvent control organisms was 0.43 and 0.85 mg, respectively. A significant difference in dry weight was detected between the control and the solvent control organisms. "ASTM states that 'If a statistical difference in either survival or growth is detected between the two controls, only the solvent control may be used as the basis for calculation of results.' Therefore, solvent control dry weight data were used for comparisons to test levels in the statistical analysis (Table 7 [attached]). No significant difference in dry weight was detected in any test level, except for the 0.65 and 1.29 mg/l concentrations. However, it does not appear that this is compound related since there was no significant difference in dry weight in the test levels above or below The difference is likely due to biological these levels. variation."

Time to first brood at 5.74 mg ai/l was significantly increased when compared to the pooled control data. The number of young produced per adult reproduction day after 21 days was significantly reduced at the two highest exposure levels when compared to the pooled control data (Table 6, attached).

Based on survival, time to first brood, number of offspring per adult per reproductive day, and length, the MATC was >1.29 and <2.62 mg ai/l. The point estimate MATC was 1.84 mg ai/l.

During the study, the test solutions had a pH of 7.8-8.2, a DO of 4.0-8.6 mg/l, and a temperature of 19.2-21.1°C.

"The results of this study agree well with the results from the daphnia life-cycle study previously submitted to the Agency (MRID 42447801). The NOECs for this study and the previous study were 1.29 and 1.32 mg/l, respectively. The LOECs for this study and the previous study and the previous study were 2.62 and 2.60 mg/l, respectively. Therefore, the chronic toxicity of SENCOR Technical to Daphnia magna has been well established."

Good laboratory practice (GLP) compliance and quality assurance statements were included in the report indicating that the study was performed in accordance with EPA GLP regulations (40 CFR Part 160).

# 14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. <u>Test Procedure</u>: An SEP for a daphnid chronic flowthrough test is not available at this time. Therefore, the SEP for conducting *Daphnia magna* renewal life-cycle toxicity tests was used as a general guidance in this data validation process. The weaknesses observed in this study are noted as follows:

The solvent concentration was not the same in all test solutions with more solvent at higher test levels. The solvent control solution contained the maximum amount of methanol used in the test. Since methanol appeared to have an effect (growth promotion) on the daphnids, the test should have been conducted either by using the same solvent concentration at all test levels or providing a series of solvent controls containing the same solvent concentrations as those present in each test level (i.e., there would have been 5 solvent controls in this test). In this test, only the highest test level solution contained the same amount of solvent as that present in the solvent control Therefore, it is not reasonable to compare solution. data from the four lower test levels to those of the solvent control.

The authors stated that water quality parameters (pH, DO, conductivity, hardness, and alkalinity) were recorded on days 0, 7, 14, and 21; however, hardness, alkalinity, and conductivity measurements were not reported.

Statistical Analysis: The reviewer used Toxstat В. (Version 3.1) computer program to compare the survival data and reproduction data (number of young/adult reproduction day and time to first brood) of the test concentrations to the controls. Since the survival and time to first brood data failed the assumptions of normality or homogeneity of variance, a non-parametric test (Kruskal-Wallis) was used to analyze the data. This analysis demonstrated no significant difference in survival between the controls and any treatment levels (page 19 of printouts, attached). Time to first brood was affected at the highest concentration compared to the controls (page 24 of printouts, attached). reproduction data (number of young per reproduction day) met assumptions of homogeneity of variance and normality; therefore, William's test was used to analyze the data (page 28 of printouts, attached). results illustrated a significant difference at the two highest test concentrations when compared to the dilution water control. These results are the same as the authors'.

Individual length and weight data were analyzed using a one-way analysis of variance (ANOVA) coupled with Bonferroni's test for treatment comparisons (printouts, attached). When compared to the solvent control, weight was significantly reduced at all test levels except the highest test concentration and lowest concentration. Length was not affected at any test level. When compared to the dilution water control, weight was greater at all test levels, although not significantly. Length was not affected at any level.

c. <u>Discussion/Results</u>: Although reduction in dry weight at the highest and lowest test concentrations were not statistically significant when compared to the solvent control, a treatment related effect at this level is apparent, since mean dry weight was 0.85 mg in the solvent control, 0.76 mg in the highest concentration, and 0.66 mg in the lowest test concentration. The reviewer concludes that the dry weight of *Daphnia magna* was affected at all exposure concentrations of SENCOR Technical. However, the dry weights for the solvent control are significantly greater than those of the

MRID No. 427312-01

dilution water control. There were no significant effects on dry weight at any test level compared to the dilution water control. Therefore, no reliable NOEC for dry weight was determined by this study. The NOEC for all other parameters (survival, time to first brood, length and number offspring per adult per day) was 1.29 mg/L.

This study is scientifically sound and meets the guideline requirements for a freshwater invertebrate life-cycle toxicity test.

# D. Adequacy of the Study:

- (1) Classification: Core.
- (2) Rationale: N/A
- (3) Repairability: N/A
- 15. COMPLETION OF ONE-LINER: Yes; December 13, 1993.

TITLE: Metripud...
metridap.dat Metribuzin daphnia life cycle dry weight stats

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATI	ON REP	VALUE	TRANS VALUE
1	solvent contr	ol 1	0.9400	0.9400
1	solvent contr		0.7800	0.7800
1	solvent contr		0.8200	0.8200
2	0.		0.6500	0.6500
2	0.		0.6000	0.6000
2	0.	30 3	0.7200	0.7200
	0.	30 4	0.6700	0.6700
2 3	0.		0.8300	0.8300
3	0.		0.4600	0.4600
3	0.		0.4300	0.4300
<sup>°</sup> 3	0.	65 4	0.5400	0.5400
4	1.		0.5300	0.5300
4	1.	29 1 29 2	0.6000	0.6000
4	1.	29 3	0.6400	0.6400
4	1.	29 4	0.6900	0.6900
4 5		62 1	0.6600	0.6600
5	2.		0.5900	0.5900
5	2.		0.6400	0.6400
5	2.		0.6400	0:6400
6	5 <b>.</b>		0.8000	0.8000
6	5.		0.7400	0.7400
6		74 3	0.7300	0.7300

Metribuzin daphnia life cycle dry weight stats File: metridap.dat Transform: NO TRANSFORMATION

### SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN	
1	solvent control	3	0.780	0.940	0.847	
2	0.30	4	0.600	0.720	0.660	
3	0.65	4	0.430	0.830	0.565	
4	1.29	4	0.530	0.690	0.615	
5	2.62	4	0.590	0.660	0.633	
6	5.74	3	0.730	0.800	0.757	

Metribuzin daphnia life cycle dry weight stats Transform: NO TRANSFORMATION File: metridap.dat

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

1	solvent control	0.007	0.083	0.048	e de la personal de la companya de l
2	0.30	0.002	0.050	0.025	
3	0.65	0.033	0.183	0.091	
4	1.29	0.005	0.068	0.034	
5	2.62	0.001	0.030	0.015	
6	5.74	0.001	0.038	0.022	

Metribuzin daphnia life cycle dry weight stats

File: metridap.dat Transform: NO TRANSFORMATION

# ANOVA TABLE

•						
SOURCE	DF	ss	MS	<b>F</b>		
Between	5	0.178	0.036	4.000		
Within (Error)	16	0.141	0.009			
Total	21	0.319				

Critical F value = 2.85 (0.05,5,16) Since F > Critical F REJECT Ho:All groups equal

Metribuzin daphnia life cycle dry weight stats

File: metridap.dat Transform: NO TRANSFORMATION

BONFERRONI T-TEST -	RONI T-TEST - TABLE 1 OF 2 Ho:C			ontrol <treatment< th=""></treatment<>		
IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG		
solvent control	0.847	0.847				
0.30	0.660	0.660	2.576			
0.65	0.565	0.565	3.887	*		
1.29	0.615	0.615	3.197	*		
2.62	0.633	0.633	2.956	*		
5.74	0.757	0.757	1.162			
	IDENTIFICATION  solvent control 0.30 0.65 1.29 2.62	TRANSFORMED  IDENTIFICATION MEAN  solvent control 0.847 0.30 0.660 0.65 0.565 1.29 0.615 2.62 0.633	TRANSFORMED MEAN CALCULATED IN ORIGINAL UNITS  solvent control 0.847 0.847 0.30 0.660 0.660 0.65 0.565 0.565 1.29 0.615 0.615 2.62 0.633 0.633	TRANSFORMED MEAN CALCULATED IN ORIGINAL UNITS T STAT  solvent control 0.847 0.847 0.30 0.660 0.660 2.576 0.65 0.565 0.565 3.887 1.29 0.615 0.615 3.197 2.62 0.633 0.633 2.956		

Bonferroni T table value = 2.58 (1 Tailed Value, P=0.05, df=16,5)

Metribuzin daphnia life cycle dry weight stats
File: metridap.dat Transform: NO TRANSFORMATION File: metridap.dat

	BONFERRONI T-TEST -	TABLE	2 OF 2	Ho: Contr	ol <treatment< th=""></treatment<>
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1 2	solvent control 0.30	3 4	0.187	22.1	0.187

3	0.65	4	0.187	22.1	0.282
4	1.29	4	0.187	22.1	0.232
5	2.62	4	0.187	22.1	0.214
6	5.74	3	0.200	23.6	0.090

TITLE:

metribuzin daphnia dry weight vs neg control

FILE:

metdap2.dat

TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	control	1	0.4800	0.4800
ī	control	2	0.4500	0.4500
1	control	3	0.3600	0.3600
ī	control	4	0.4400	-0.4400
2	0.30	1	0.6500	0.6500
2	0.30	2	0.6000	0.6000
2	0.30	3	0.7200	0.7200
2	0.30	4	0.6700	0.6700
. 3	0.65	1	0.8300	0.8300
3	0.65	1 2	0.4600	0.4600
3	0.65	3	0.4300	0.4300
3	0.65	4	0.5400	0.5400
4	1.29	1	0.5300	0.5300
4	1.29	2	0.6000	0.6000
4	1.29	3	0.6400	0.6400
4	1.29	4	0.6900	0.6900
5	2.62	1	0.6600	0.6600
5 5	2.62	2	0.5900	0.5900
5	2.62	3	0.6400	0.6400
5	2.62	4	0.6400	0:6400
6	5.74	1	0.8000	0.8000
6	5.74	2	0.7400	0.7400
6	5.74	3	0.7300	0.7300

metribuzin daphnia dry weight vs neg control Transform: NO TRANSFORMATION File: metdap2.dat

### SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	0.360	0.480	0.433
2	0.30	4	0.600	0.720	0.660
3	0.65	4	0.430	0.830	0.565
4	1.29	4	0.530	0.690	0.615
5	2.62	4	0.590	0.660	0.633
6	5.74	3	0.730	0.800	0.757

metribuzin daphnia dry weight vs neg control

File: metdap2.dat Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	and the second of the second o
1	control	0.003	0.051	0.026	
2	0.30 0.65	0.002 0.033	0.050 0.183	0.025 0.091	
3 4	1.29	0.005	0.068	0.034	
5	2.62	0.001	0.030	0.015	
6	5.74	0.001	0.038	0.022	

metribuzin daphnia dry weight vs neg control File: metdap2.dat Transform: NO TRANSFORMATION

#### ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.210	0.042	5.250
Within (Error)	17	0.135	0.008	
Total	22	0.345		

Critical F value = 2.81 (0.05,5,17) Since F > Critical F REJECT Ho:All groups equal

metribuzin daphnia dry weight vs neg control

Transform: NO TRANSFORMATION File: metdap2.dat

	BONFERRONI T-TEST -	TABLE 1 OF 2	Ho: Control <treatment< th=""></treatment<>		
GROU	P IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.433	0.433		
2	0.30	0.660	0.660	-3.597	
3	0.65	0.565	0.565	-2.095	
4	1.29	0.615	0.615	-2.886	
5	2.62	0.633	0.633	-3.162	
6	5.74	0.757	0.757	-4.745	

Bonferroni T table value = 2.57 (1 Tailed Value, P=0.05, df=17,5)

metribuzin daphnia dry weight vs neg control
File: metdap2.dat Transform: NO TRANSFORMATION File: metdap2.dat

	BONFERRONI T-TEST -	- TABLE	2 OF 2	Ho: Contr	ol <treatment< th=""></treatment<>
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4		· · · · · · · · · · · · · · · · · · ·	

2	0.30	4	0.162	37.5	-0.228
3	0.65	4	0.162	37.5	-0.133
4	1.29	4	 0.162	37.5	-0.183
5	2.62	4	0.162	37.5	-0.200
6	5.74	3	0.175	40.5	-0.324

TITLE: Metribuzin daphnid length vs pooled control metlngth.dat

TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIF:	ICATION	REP	; 7	ALUE	TRAN	S VALUE	•
								*
1	pooled co	ontrols	1		4.3000		4.3000	
1	pooled co		2	4	4.0000		4.0000	
1	pooled co		3	*46	4.1000		4.1000	
1	pooled co		4		4.2000		4.2000	
1	pooled co		5		4.5000		4.5000	
1	pooled co		6		4.4000	# # # # # # # # # # # # # # # # # # #	4.4000	
1	pooled co		7	-	4.2000	_	4.2000	•
2		0.30		·	4.3000		4.3000	
. 2 2 2		0.30	1 2 3		4.2000		4.2000	
2		0.30	3		4.3000		4.3000	
2		0.30	4		4.3000		4.3000	
3		0.65	1		4.5000		4.5000	
3		0.65	2		4.3000		4.3000	
		0.65	3		4.2000		4.2000	
3 3		0.65	4		4.2000		4.2000	
4		1.29	1		4.4000		4.4000	
4		1.29	2		4.1000		4.1000	•
4		1.29	3		4.4000	•	4.4000	ř.
4		1.29	4		4.4000		4.4000	4
		2.62	1		4.3000		4.3000	
5 5		2.62	2		4.2000		4.2000	
5		2.62	3		4.2000		4.2000	
.5		2.62	4		4.2000		4.2000	
6		5.74	i		4.4000		4.4000	
6		5.74	2		4.3000		4.3000	
6		5.74	3	-	4.4000		4.4000	

Metribuzin daphnid length vs pooled control

File: metlngth.dat Transform: NO TRANSFORMATION

## SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	pooled controls	7	4.000	4.500	4.243
2	0.30	4	4.200	4.300	4.275
3	0.65	4	4.200	4.500	4.300
4	1.29	4	4.100	4.400	4.325
5	2.62	4	4.200	4.300	4.225
6	5.74	3	4.300	4.400	4.367

Metribuzin daphnid length vs pooled control File: metlngth.dat Transform: NO TRANSFORMATION

### SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

·	SEM	SD	VARIANCE	IDENTIFICATION	GRP
	0.065	0.172	0.030	pooled controls	1
	0.025	0.050	0.003	0.30	2
	0.071	0.141	0.020	0.65	.3
	0.075	0.150	0.023	1.29	4
	0.025	0.050	0.003	2.62	,5
	0.033	0.058	0.003	5.74	6.

Metribuzin daphnid length vs pooled control

File: metlngth.dat Transform: NO TRANSFORMATION

### ANOVA TABLE

SOURCE	DF	ss	MS	F
Between	5	0.054	0.011	0.688
Within (Error)	20	0.326	0.016	
Total	25	0.380		

Critical F value = 2.71 (0.05,5,20) Since F < Critical F FAIL TO REJECT Ho:All groups equal

Metribuzin daphnid length vs pooled control

File: metlngth.dat Transform: NO TRANSFORMATION

	BONFERRONI T-TEST -	TABLE 1 OF 2	Ho: Contro	l <treatment< th=""><th>:</th></treatment<>	:
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT SI	.G
1 2	pooled controls 0.30	4.243 4.275	4.243 4.275	-0.405	-
3 4	0.65 1.29	4.300 4.325	4.300 4.325	-0.721 -1.036	
5 6	2.62 5.74	4.225 4.367	4.225 4.367	0.225 -1.418	

Bonferroni T table value = 2.53 (1 Tailed Value, P=0.05, df=20,5)

Metribuzin daphnid length vs pooled control
File: metlngth.dat Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho: Control<Treatment

GROUP	IDENTIFICATION	REPS	(IN ORIG. UNITS)	CONTROL	FROM CONTROL
1 2 3 4 5 6	pooled controls 0.30 0.65 1.29 2.62 5.74	7 4 4 4 4 3	0.200 0.200 0.200 0.200 0.200 0.221	4.7 4.7 4.7 4.7 5.2	-0.032 -0.057 -0.082 0.018 -0.124
					0.124

Metribuzin daphnid length vs pooled control File: metlngth.dat Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	pooled controls	7	4.243	4.243	4.243
2	0.30	4	4.275	4.275	4.275
3	0.65	4	4.300	4.300	4.283
4	1.29	4	4.325	4.325	4.283
5	2.62	4	4.225	4.225	4.283
6	5.74	3	4.367	4.367	4.367

Metribuzin daphnid length vs pooled control File: metlngth.dat Transform: NO TRANSFORMATION

WILLIAMS TEST	(Isotonic	regression	model)	TABLE 2 O	F 2
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
pooled controls 0.30 0.65 1.29 2.62 5.74	4.243 4.275 4.283 4.283 4.283 4.367	0.401 0.506 0.506 0.506 1.404		1.72 1.81 1.83 1.85 1.86	k= 1, v=20 k= 2, v=20 k= 3, v=20 k= 4, v=20 k= 5, v=20

s = 0.128

Note: df used for table values are approximate when v > 20.

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	Identity of product inert ingredients.
<del></del>	Identity of product impurities.
	Description of the product manufacturing process.
	Description of quality control procedures.
	Identity of the source of product ingredients.
	Sales or other commercial/financial information.  A draft product label.
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SENCOR: Survival of Exposed Daphnia magna
File: 42731201.sur Transform: ARC SINE(SQUARE ROOT(Y))

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.742	6.292	9.932	6.292	1.742
OBSERVED	0	5	18	3	0

Calculated Chi-Square goodness of fit test statistic = 12.0255 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

SENCOR: Survival of Exposed Daphnia magna

File: 42731201.sur Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption. Additional transformations are useless.

TITLE: SENCOR: Survival of Exposed Daphnia magna FILE: 42731201.sur
TRANSFORM: NO TRANSFORM NUMBER OF GRO

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE	
1	Control	1	1.0000	1.0000	
1	Control	2	1.0000	1.0000	
1	Control	3	1.0000	1.0000	
1	Control	4	1.0000	1.0000	
2	Solvent Control	. 1	0.9000	0.9000	7
2	Solvent Control	2	1.0000	1.0000	
2	Solvent Control	3 1	1.0000	1.0000	
3	0.30  mg/l	1	0.8000	0.8000	
٠ 3	0.30  mg/l	2	0.9000	0.9000	
3	0.30  mg/l	3	1.0000	1.0000	
3	0.30  mg/l	4	0.9000	0.9000	
4	0.65  mg/l	1	1.0000	1.0000	1
4	0.65  mg/l	1 2 3	0.9000	0.9000	
4	0.65  mg/l		1.0000	1.0000	
4	0.65  mg/l	4	1.0000	1.0000	
5	1.29  mg/l	1	1.0000	1.0000	
5	1.29  mg/l	2	1.0000	1.0000	
5	1.29  mg/l	3	0.8000	0.8000	
.5	1.29  mg/l	4	1.0000	1.0000	
6	2.62  mg/l	1	1.0000	1.0000	
6	2.62  mg/l	2	0.9000	0.9000	
6	2.62  mg/l	3	1.0000	1.0000	
6	2.62  mg/l	4	1.0000	1.0000	
7	5.74  mg/l	1	1.0000	1.0000	
7	5.74  mg/l	.2	1.0000	1.0000	
7	5.74 mg/l	. 3	1.0000	1.0000	

SENCOR: Survival of Exposed Daphnia magna

Transform: NO TRANSFORM File: 42731201.sur

## KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	1.000	1.000	68.000
2	Solvent Control	0.967	0.967	39.000
3	0.30  mg/l	0.900	0.900	28.500
4	0.65  mg/l	0.975	0.975	56.000
5	1.29  mg/l	0.950	0.950	52.500
6	2.62 mg/l	0.975	0.975	56.000
7	5.74 mg/l	1.000	1.000	51.000

Calculated H Value = 7.132 Critical H Value Table = 12.590 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

SENCOR: Survival of Exposed Daphnia magna

File: 42731201.sur Transform: NO TRANSFORM

## DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 3 5 2 4 6 1 7
3 5 2 4 6 1 7	0.30 mg/l 1.29 mg/l Solvent Control 0.65 mg/l 2.62 mg/l Control 5.74 mg/l	0.900 0.950 0.967 0.975 0.975 1.000	0.900 0.950 0.967 0.975 0.975 1.000	

Table q value (0.05,7) = 3.038

<sup>\* =</sup> significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 Unequal reps - several SE values used

SENCOR: Time to 1st Brood of Exposed Daphnia magna

File: 42731201.tfb Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	1	27	0	0

Calculated Chi-Square goodness of fit test statistic = 40.3039 Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

SENCOR: Time to 1st Brood of Exposed Daphnia magna File: 42731201.tfb Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.750

V = 0.434

Critical W (P = 0.05) (n = 28) = 0.924Critical W (P = 0.01) (n = 28) = 0.896

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

SENCOR: Time to 1st Brood of Exposed Daphnia magna File: 42731201.tfb Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption. Additional transformations are useless.

TITLE:

SENCOR: Time to 1st Brood of Exposed Daphnia magna 42731201.tfb

TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE	
1	Control	1	8.0000	8.0000	
1	Control	2	8.0000	8.0000	
1	Control	3	8.0000	8.0000	
1	Control	4	8.0000	8.0000	
2	Solvent Control	1	8.0000	8.0000	
2	Solvent Control	2	8.0000	8.0000	
2	Solvent Control	.3	8.0000	8.0000	
. 2	Solvent Control	. 4	8.0000	8.0000	
3	0.30  mg/l	1	8.0000	8.0000	
. 3	0.30  mg/l	2	8.0000	8.0000	
3	0.30  mg/l	3	8.0000	8.0000	
3 3	0.30  mg/l	4	8.0000	8.0000	: .
4	0.65  mg/l	1	8.0000	8.0000	
4	0.65  mg/l	2	8.0000	8.0000	
4	0.65  mg/l	3	8.0000	8.0000	
4 5	0.65  mg/l	4	8.0000	8.0000	
5	1.29  mg/l	1	8.0000	8.0000	
5 5	1.29  mg/l	2	8.0000	8.0000	
5	1.29  mg/l	3	8.0000	8.0000	
5	1.29  mg/l	4	8.0000	8.0000	
6	2.62  mg/l	1 2	8.0000	8.0000	
6	2.62  mg/l	2	8.0000	8.0000	
6	2.62  mg/l	3	8.0000	8.0000	
6	2.62  mg/l	4	8.0000	8.0000	
7	5.74  mg/l	1	* 8.0000	8.0000	
7	5.74  mg/l	2 3	9.0000	9.0000	
7	5.74  mg/l	3	9.0000	9.0000	
7	5.74 mg/l	4	9.0000	9.0000	

SENCOR: Time to 1st Brood of Exposed Daphnia magna

Transform: NO TRANSFORMATION File: 42731201.tfb

## KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	Control	8.000	8.000	52.000
2	Solvent Control	8.000	8.000	52.000
3	0.30  mg/l	8.000	8.000	52.000
4	0.65  mg/l	8.000	8.000	52.000
5	1.29 mg/l	8.000	8.000	52.000
6	2.62  mg/l	8.000	8.000	52.000
7	5.74 mg/l	8.750	8.750	94.000

Calculated H Value = 19.440 Critical H Value Table = 12.590 Since Calc H > Crit H REJECT Ho: All groups are equal.

SENCOR: Time to 1st Brood of Exposed Daphnia magna

File: 42731201.tfb Transform: NO TRANSFORMATION

## DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

			P			GI	ROI	JP						
		TRANSFORMED	ORIGINAL	0	0	0	0	0	0	0				7
GROUP	IDENTIFICATION	MEAN	MEAN	2	3	4	5	6	,1	7				•
				-	_	***	_	_		ı —				
2	Solvent Control	8.000	8.000	1										
3	0.30  mg/l	8.000	8.000	•	/						17			
4	0.65  mg/l	8.000	8.000	•	•	1								
5	1.29  mg/l	8.000	8.000		.,•	•	1							
6	2.62 mg/l	8.000	8.000		•	•	•	1						
1	Control	8.000	8.000	•	•	•	•	•	/					
7	5.74  mg/l	8.750	8.750	*	*	*	*	*	*	/				

<sup>\* =</sup> significant difference (p=0.05) Table q value (0.05,7) = 3.038

<sup>. =</sup> no significant difference
SE = 3.118

SENCOR: # Young/Reproductive Day of Exposed D. magna File: 42731201.rep Transform: NO TRANSFORMATION

				The state of the s	_		
		£		~~+11~1	222	AVNACTAC	frequencies
Chi-smiare	Test	TOL	normatity:	actual	anu	expedied	Treductiones
CIII GGGGEC							<del>-</del>

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED OBSERVED	1.742	6.292 9	9.932	6.292	1.742
•					

Calculated Chi-Square goodness of fit test statistic = 4.7296 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

SENCOR: # Young/Reproductive Day of Exposed D. magna Transform: NO TRANSFORMATION File: 42731201.rep

Bartletts test for homogeneity of variance

Calculated B statistic = 15.22

Table Chi-square value = 16.81 (alpha = 0.01) Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) =

Used for Chi-square table value ==> df (#groups-1) = 6 

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

TITLE: SENCOR: # Young/Reproductive Day of Exposed D. magna FILE: 42731201.rep
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE	
1	Control	1	6.8800	6.8800	
1	Control	2	5.4500	5.4500	
1	Control	. 3	6.1800	6.1800	٠
1	Control	4	5.3400	5.3400	
2	Solvent Control	1	5.4500	5.4500	
2	Solvent Control	2	5.3100	5.3100	
2	Solvent Control	3 1 2 3	5.0700	5.0700	
3	0.30  mg/l	1	7.7100	7.7100	
· 3	0.30  mg/l	2	5.3200	5.3200	
3	0.30  mg/l	3	5.4700	5.4700	
3	0.30  mg/l	. 4	6.2700	6.2700	
4	0.65  mg/l	1	6.2700	6.2700	
4	0.65  mg/l	2	5.2500	5.2500	
4	0.65  mg/l	3	6.4100	6.4100	
4	0.65  mg/l	4 1 2 3	6.1400	6.1400	
5	1.29  mg/l	1	6.5200	6.5200	
5	1.29  mg/l	2	, 5 <b>.</b> 1600	5.1600	
5 5	1.29  mg/l		6.1300	6.1300	
5	1.29  mg/l	4 1	6.0200	6.0200	
6	2.62  mg/l	1	3.1800	3.1800	
6	2.62  mg/l	2 3	3.1500	3.1500	
6	2.62  mg/l	. 3	4.2600	4.2600	•
6	2.62  mg/l	4	3.0800	3.0800	
7	5.74  mg/l	4 1 2	1.0800	1.0800	
7	5.74  mg/l	2	1.0300	1.0300	
7	5.74 mg/l	3	0.9700	0.9700	

SENCOR: # Young/Reproductive Day of Exposed D. magna File: 42731201.rep Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	4	5.963	5.963	5.963
2	Solvent Control	3	5.277	5.277	5,900
. 3	0.30  mg/l	4	6.192	6.192	5.900
4	0.65  mg/l	4	6.018	6.018	5.900
5	1.29  mg/l	4	5.957	5.957	5.900
6	2.62 mg/l	4	3.418	3.418	3.418
7	5.74 mg/l	3	1.027	1.027	1.027

SENCOR: # Young/Reproductive Day of Exposed D. magna File: 42731201.rep Transform: NO TRANSFORMATION

	WILLIAMS TEST	(Isotonic	regression	model)	TABLE 2 O	F 2
, <b></b>	IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
	Control	5.963	*			
	Solvent Control	5.900	0.126		1.73	k=1, v=19
	0.30  mg/l	5.900	0.136	9	1.81	k=2, v=19
	0.65  mg/l	5.900	0.136		1.84	k = 3, v = 19
	1.29  mg/l	5.900	0.136		1.85	k = 4, v = 19
	2.62 mg/l	3.418	5.557	*	1.86	k=5, v=19
	5.74  mg/l	1.027	9.978	*	1.87	k = 6, v = 19

s = 0.648

Note: df used for table values are approximate when  $\dot{v}$  > 20.

Ecological Effects Branch One-Liner Data Entry Form

Chemical Metribuzen

Shaughnessy No. 101101

Pesticide Use

INVERTEBRATE ACUTE TOXICITY	AI AI	EC <sub>50</sub> (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								3
3.		*		,				
4.								
5.			,		,		•	
• 9								
7.					C.			
CHRONIC TOX.	%I	MATC LC <sub>50</sub>	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.Daphnia magna	93	ND >5.74 mg ai/1	21	. Weight	1993/1993	42731201 Supple- mental	MI	RGM
2.	Þ							
3.								,

MI=Miles Incorporated COMMENTS: Based on mean measured concentrations.